CRYPTOSPORIDIOSIS AMONG BIRDS AND BIRD HANDLERS AT ZOO NEGARA, KUALA LUMPUR

MUHAMAT SHUKRI MAHAT

DEPARTMENT OF MOLECULAR MEDICINE
FACULTY OF MEDICINE
UNIVERSITY OF MALAYA
KUALA LUMPUR
2006 / 2007

CRYPTOSPORIDIOSIS AMONG BIRDS AND BIRD HANDLERS AT ZOO NEGARA, KUALA LUMPUR

MUHAMAT SHUKRI MAHAT

DEPARTMENT OF MOLECULAR MEDICINE
FACULTY OF MEDICINE
UNIVERSITY OF MALAYA
KUALA LUMPUR
2006 / 2007

A research report submitted to the Department of Molecular Medicine, Faculty of Medicine, University of Malaya, in partial fulfillment of the requirements for the degree of Bachelor of Biomedical Sciences 2006/07

ACKNOWLEDGEMENTS

Alhamdulillah, at last I managed to finish all the laboratory experiments and my thesis writing on "Cryptosporidiosis among birds and bird handlers at Zoo Negara, Kuala Lumpur."

First and foremost, I would like to express my sincere gratitude to my understanding supervisors, Dr. Yvonne Lim Ai Lian and Professor Dr. Rohela Mahmud, for their excellent supervision, support, advice and encouragement given while completing my project and writing of this thesis.

Next, I want to thank Dr. Mohamad Ngah, Director of the Zoo Negara who gave me permission to collect the fecal samples of birds and bird handlers at Zoo Negara.

Many thanks also go to Dr. S Vellayan, Assistant Director Veterinary Services, Zoo Negara, Miss Doreen Khoo, the Bird Curator and also to all the staff of Bird Department for assisting me in the identification of birds and collection of fecal specimens for my study.

My gratitude to all academic and non-academic staff of the Department of Molecular Medicine especially Associate Professor Dr. Khalifah Sidek and Associate Professor Dr. Mary Anne for their help, advice and support given throughout my study. Besides that, I would also like to thank all staff of the Department of Parasitology especially Miss Hasidah, Mr Hafiz, Mr Redwan and Mr Yap for providing me with the materials and equipments needed.

And last but not least, I would like to thank my family and friends for giving me a lot of encouragement and moral support.

TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES PAGE

ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
°C	Degree celcius
et al.	et alia (and others)
µm	micrometer
ml	milliliter
%	percent
spp.	Species
C. muris	*Cryptosporidium muris*

CHAPTER 1: ABSTRACT

1.0 ABSTRACT

This study was carried out at the Malaysian National Zoo to ascertain not only the current prevalence rate in the birds but also to determine the association between cryptosporidiosis in birds and the bird handlers. 116 fecal samples from 71 species of birds were collected from six different locations in Zoo Negara and 8 fecal samples from bird handlers were also sampled. Results showed that the prevalence of *Cryptosporidium* oocysts in bird and bird handlers were 3.4% (4 out of 116 samples) and 12.5% (1 out of 8 samples) respectively. The birds that were positive for cryptosporidiosis were Wreathed Hornbill (*Aceros undulates)* and Great Currasaw *(Crax rubra)* from the aviary, Bushy-crested Hornbill (*Anorrhinus galeritus*) from the bird house and Common Peafowl (*Pavo Cristatus)* from the lake. Birds at the lake showed the highest percentage (12.5%) of being positive followed by birds at the aviary (5.4%) and birdhouse (2.8%). Results of the present study seemed to indicate that cryptosporidiosis might be spreading to other species of birds (a comparison with a similar study conducted in 2004) and to other locations in the zoo which was not previously documented. This study also discovered the probable association of cryptosporidiosis among birds and bird handlers. However, conclusive remarks can only be done after the confirmation of speciation found in birds and bird handlers through molecular identification.

CHAPTER 2: INTRODUCTION

2.0 INTRODUCTION

2.1 *Cryptosporidium spp.*

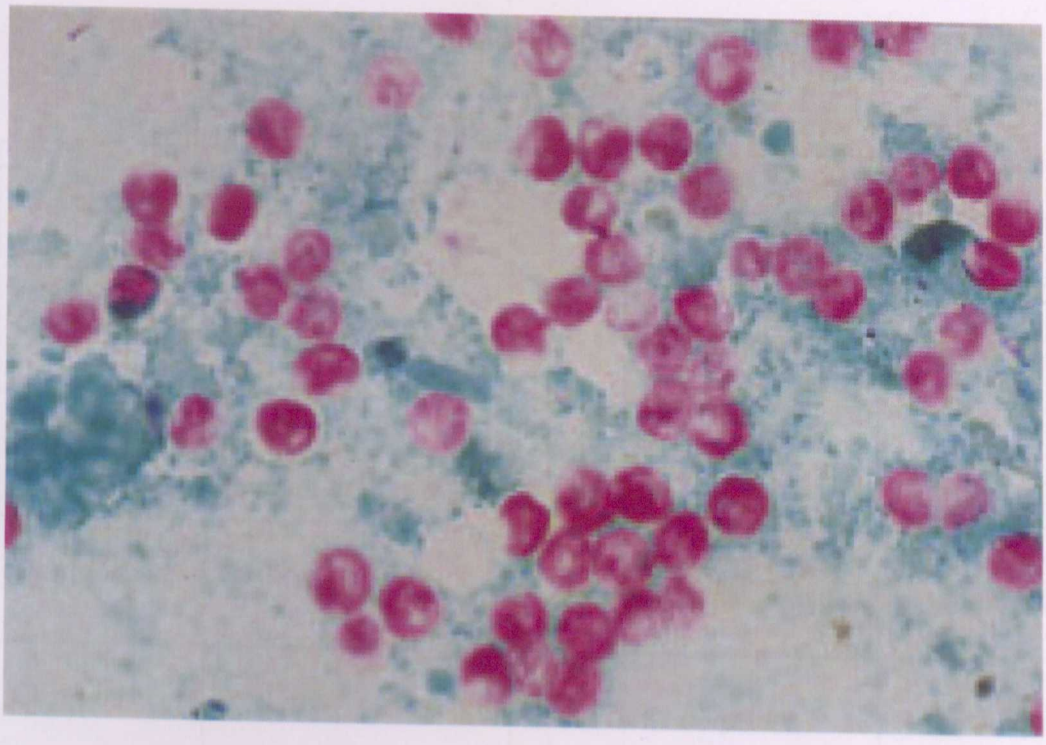
Cryptosporidium is a ubiquitous enteric protozoan pathogen that infects humans, domestic and feral animals, worldwide. It is an important causative agent of diarrhea diseasel in man, leading to significant morbidity and mortality in both developing and developed countries. *Cryptosporidium* is classified as eukaryote in the phylum Apicomplexa. It is an obligate, intracellular protozoa parasite that undergoes development culminating in the production of an encysted stage is the feces of the host. The oocyst is the primary importance for the dispersal, survival and infectivity of the parasite. Because of that, the oocysts are the stage of major importance for detection and identification of the parasite. The oocysts measure between 4 to 6 μm in diameter (Fayer *et al*, 2000) (Figure 1).

E.E. Tyzzer, an American parasitologist, has been acknowledged as the first to discover this novel protozoan parasite in the gastric epithelium of asymptomatic laboratory mice and Japanese waltzing mice at the turn of the century (in 1907). The small parasites were considered to represent a new genus of coccidia-like sporozoa which was eventually named *Cryptosporidium* (meaning hidden sporocysts in Greek) because, unlike other coccidia, the transmissive phase of the parasite, the oocyst, did not have sporozoites enveloped by sporocysts within the oocyst. The species was later called *Cryptosporidium muris*. About five years later, Tyzzer (1912) once again observed similar parasites resembling *C. muris*, however this time, they were

smaller in size and occurred in the small intestines of laboratory mice and rabbits.

These parasites were considered to be a separate species (hence the name *Cryptosporidium parvum*) because of the difference in oocysts size as compared with *C. muris.* Besides that, their development was confined to the small intestinal epithelium and not the gastric epithelium as in *C. muris* (Sterling and Arrowood, 1978). Tyzzer (1912) reported that both the gastric (*C. muris*) and the intestinal (*C. parvum*) species may occur in the same individual under ordinary laboratory conditions, but either is frequently present in great numbers in the absence of the other.

Currently, there are 16 species of *Cryptosporidium* which have been identified having different morphologies and hosts (Xiao *et al*, 2004)(Table 1).



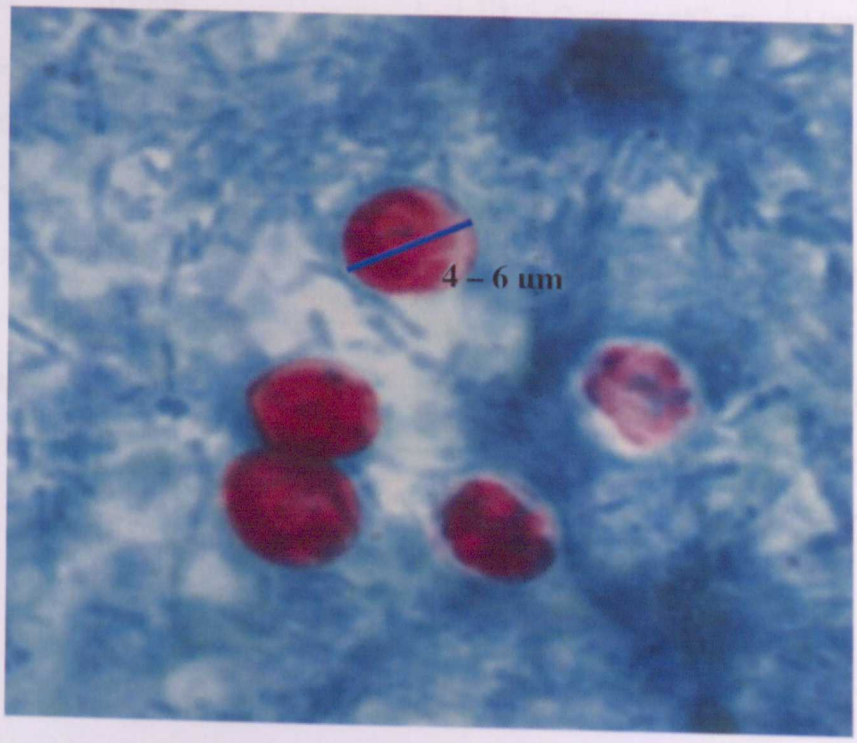


Figure 1: *Cryptosporidium* oocysts

Table 1: *Cryptosporidium* species and their respective host

No	Species	Hosts	No	Species	Hosts
1	*C. hominis*	Humans, monkeys	9	*C. baileyi*	Poultry
2	*C. parvum*	Cattle, other ruminants,humans	10	*C. meleagridis*	Turkeys, humans
3	*C.andersoni*	Cattle	11	*C. galli*	Finches, chicken
4	*C. muris*	Rodents	12	*C. serpentis*	Reptiles
5	*C. suis*	Pigs	13	*C. saurophilum*	Lizard
6	*C. felis*	Cats	14	*C. molnari*	Fish
7	*C. canis*	Dogs	15	*C. bovis*	Cattle, sheep
8	*C. wrairi*	Guinea pigs	16	*C. scophthalmi*	Fish

2.2 Cryptosporidiosis

2.2.1 Avian cryptosporidiosis

The first avian species of *Cryptosporidium* was described in 1929 by Tyzzer from the caeca of chicken. Then in 1955, Slavin described a similar spesies from turkeys poults and named it *Cryptosporidium meleagridis* (Morgan *et al,* 2001). In 1986, Current, Upton and Haynes isolated and described an organism from chickens and gave the name *C. baileyi* to the species.

Cryptosporidium species that have been described in birds include *C. meleagridis*, *C. baileyi* and *C. galli* (Awad-el-Kariem *et al*, 1997). Only *C. meleagridis*, which infects turkeys and parrot, is a known threat to human beings. Nevertheless, *C. baileyi* is probably the most common avian *Cryptosporidium* species since it is able to infect chickens, turkeys, ducks, cockatiels, quails and ostriches whereas *C.galli*, the latest addition to the family infects hosts such as finches, domestic chickens, capercaille and pine grosbeaks (Xiao *et al,* 2004).

In birds, *Cryptosporidium sp.* has not only been reported in chickens (Sterling and Arrowood, 1978), turkeys (Srèter and Varga, 1999), quails, pheasants, peafowls, junglefowls, ducks, geese, parrots, pinches, lovebirds, budgerigars, ostriches (Morgan *et al*, 2001), catercaille, pine grosbeaks (Xiao *et al*, 2004), but the latest addition to this list is the wrinkled hornbill found in Malaysia (Rohela *et al*, 2005).

Avian cryptosporidiosis can manifest itself as respiratory (Dhillon *et al.* 1994) and intestinal (Lindsay and Blagburn, 1990) diseases. In some cases, cryptosporidiosis might even manifest as renal disease and may be fatal (Hoerr *et al,* 1986). In avian respiratory cryptosporidiosis, the parasite can infect the nasal turbinates, nasopharynx, sinuses, larynx, trachea, lungs, air sac and conjunctiva (Srêter and Varga, 1999). Intestinal cryptosporidiosis on the other hand, demonstrates *Cryptosporidium* in salivary and esophagus gland, proventriculus, small intestine, cecum, colon, cloaca and bursa of Fabricius (Fayer, 2004; O'Doroghue, 1995; Rohela *et* al, 2005)

The association between cryptosporidiosis in animals and animal handlers is a topic of interest. In 1981, a case of cryptosporidiosis in an animal handler was reported. The animal handler was a previously healthy 25 year old male who had symptoms of cryptosporidiosis such as nausea, low-grade fever, moderate abdominal cramps, anorexia, 5 to 10 watery, frothy bowel movements a day and constipation. Diagnosis was confirmed with the finding of *Cryptosporidium sp.* oocysts in the feces (Anonymous, 1982). Following this report, many similar cases of cryptosporidiosis were reported among animal handlers (Augus, 1983; Graczyk, 1996).

2.2.2 Cryptosporidiosis in human

Cryptosporidium infections were first reported in two human patients suffering from severe watery diarrhoea. One of the patients was an

immunocompetent 3 year old girl living in a farmhouse in Tennessee. *Cryptosporidium* was found in her rectal biopsy (Nime *et al*, 1976). The other was a 39 year old immunosuppressed man (Meisel *et al*, 1976). Even then, relatively few were subsequently diagnosed (only 12 cases in the world literature by the end of 1982) (Weber, 1985).

The organism was regarded as an opportunistic pathogen essentially, because five of the seven human cases reported between 1976 and 1981 were in immunocompromised patients (Soave & Armstrong, 1986), however, during the 1980s, cryptosporidiosis has been recognized as a common cause of acute self-limiting gastro-enteritis in immunocompetent people. In 1982, severe cryptosporidial diarrhea was reported to have occurred in 21 males who had acquired immune deficiency syndrome (AIDS) (Anonymous, 1982; Sterling and Arrowood, 1978). Since then number of recognized cases began to increase tremendously since cryptosporidiosis was reported to be a life-threatening infection in AIDS patients.

Cryptosporidiosis is a disease that is caused by *Cryptosporidium spp.* Cryptosporidiosis in humans typically manifests itself as a self-limiting disease with a median duration of 9–15 days, resulting in total recovery in healthy individuals. The major symptom is watery diarrhea associated with abdominal cramps, anorexia, weight loss, nausea, vomiting, fatigue and low-grade fever. Symptoms are similar in children and adults, although cryptosporidiosis

acquired during infancy may have permanent effects on growth and development.

However, in the immunocompromised host which is due to a variety of causes including but not limited to HIV infection and AIDS, drugs, organ transplantation, cancer chemotherapy, etc.(O'Donoghue, 1995), the infections can be prolonged and debilitating. Patients can have chronic diarrhea that can last for more than 2 months, shed oocysts in stool during the entire period, contributing to severe dehydration, weight loss and malnutrition, extended hospitalizations, and mortality. Thus, the severity and duration of illness depends on the host's immune status. The groups implicated with higher risks of infection include children and staff in day care centers, farmers and animal handlers, and health care workers. Travelers are also at risk when they travel from developed to developing countries with high prevalence of the disease.

Contaminated water and contaminated food represents the major source of *Cryptosporidium* infections for humans. Several waterborne outbreaks of cryptosporidiosis have been reported implicating contaminated drinking water and recreational water (Lionel, 2004; Walter *et al,* 2004). The most severe and largest human waterborne outbreak occurred in Milwaukee in 1993, where more than 400,000 people were infected and slightly more than 100 deaths (MacKenzie *et al*, 1994).

2.3 Transmission of *Cryptosporidium* oocysts

The oocyst is the stage transmitted from an infected host to a susceptible host by a fecal–oral route. Route of transmission can be (1) person-to-person through direct or indirect contact, possibly including sexual activities, (2) animal-to-animal, (3) animal-to-human, (4) waterborne through drinking water or recreational water, (5) food-borne, and possibly airborne (Fayer, 2000). Human usually acquire cryptosporidiosis from accidental ingestion of oocyst from contaminated water and food (Alves *et al*, 2006; Lionel, 2004; Walter *et al*, 2004).

2.4 Life Cycle

Life cycle of *Cryptosporidium spp.* was firstly described by Tyzzer which he observed in the gastric glands of laboratory mice. The life cycle consists of many stages such as excystation, merogony, gametogony, fertilization, oocyst formation, and sporogony (Sterling and Arrowood, 1978). *Cryptosporidium spp.* has homogenous life cycle in which all developmental stages (asexual and sexual) occur within one host. Once ingested, the oocysts will excyst in the gastrointestinal tract releasing the infective sporozoites (O'Donoghue; 1995, Fayer, 2004). The sporozoites immediately attach and penetrate into the host epithelial cells and develop into schizonts at the luminal surface of the epithelium.

Sporozoite will differentiate into trophozoite and then with further differentiation to become schizont that contains eight distinct merozoites. Merozoites in turn can reinitiate schizogony or enter into sexual phase of the cycle, which develop into gametocytes. The sexual reproduction occurs by gametogony and eventually both microgamonts (male) and macrogamonts (female) are formed. The fertilization of gametes will produce zygotes. Zygotes undergo further asexual development leading to the production of the thick-walled oocysts, which are oocysts, containing 4 sporozoites. The oocysts are next release in the feces and ready to infect the other organism (Sterling and Arrowood, 1978, O'Donoghue, 1995) (Figure 2).

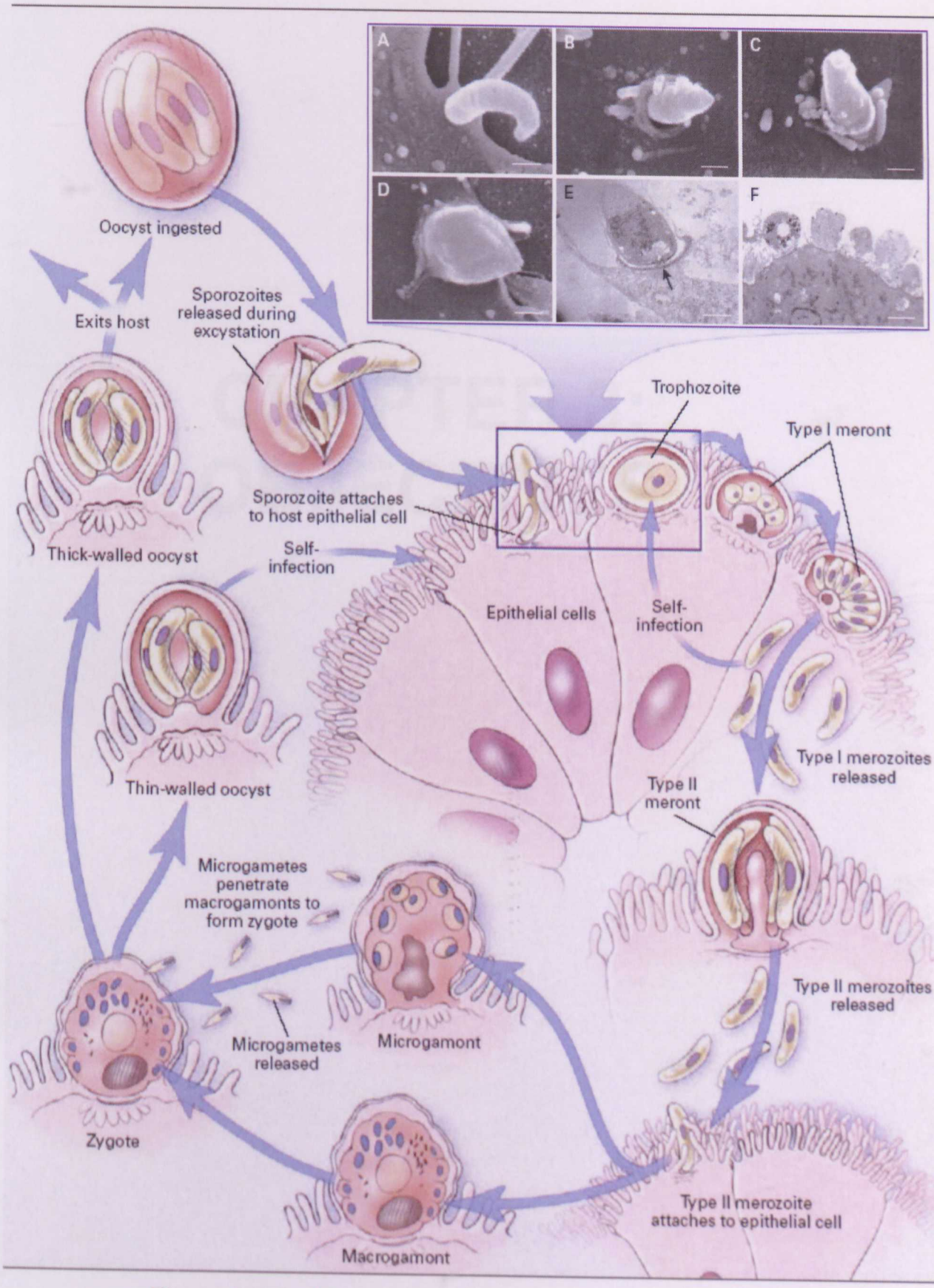


Figure 2: The life cycle of *Cryptosporidium* spp.

CHAPTER 3: OBJECTIVES

3.0 OBJECTIVES OF STUDY

This study has two main objectives which are:-

1. To re-evaluate the occurrence of *Cryptosporidium sp.* oocyst in the fecal samples of birds in the Zoo Negara, Kuala Lumpur, Malaysia.

2. To determine the prevalence of cryptosporidiosis among bird handlers and its association with the occurrence of cryptosporidiosis in birds.

CHAPTER 4: MATERIALS AND METHODS

4.0 METHODOLOGY

4.1 Location of study

The study was carried out in Kuala Lumpur National Zoo, located at Ulu Kelang, 13 km northeast of Kuala Lumpur (Figure 3). The birds at the National Zoo can be found at various places such as the aviary, bird house, breeding area, animal show, children's world and lake (Figure 4 and 5). All birds in the locations mentioned are caged except those in the lake. Birds housed in the aviary are birds of various species which include smaller birds such as Lovebirds to the larger birds such as Common Peafowl and Hornbill. Birdhouse and breeding area are places where the birds are quarantined before being sent to the aviary for visitors to view. It is also a place for the offspring of the birds. Children's World is designed especially for children to have a closer view of domesticated animals and birds such as macaws and parrots. Birds used at the Animal Show are trained to perform stunts and entertaining activities. Storks and pelicans are the types of birds commonly found in the lake where they are free to roam.

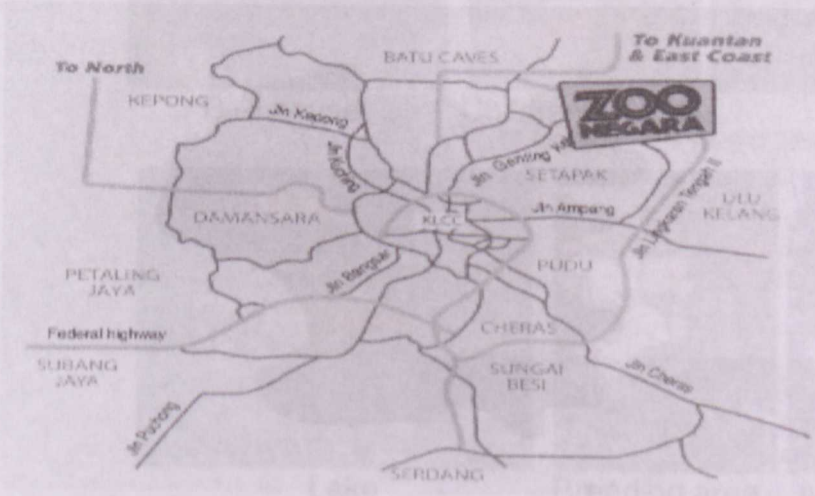


Figure 3: Location of the Zoo Negara

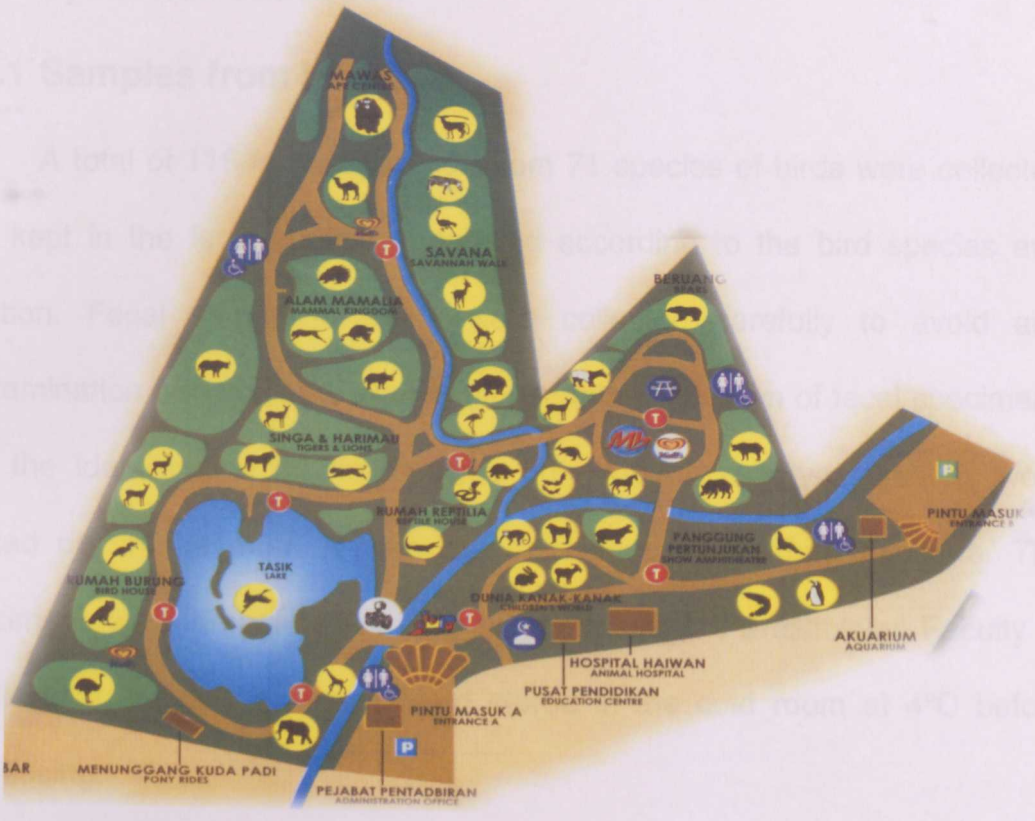
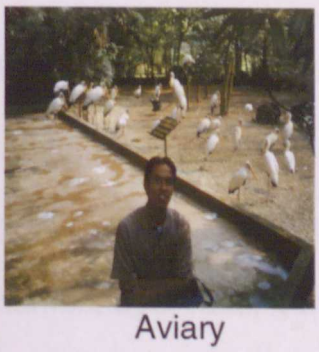


Figure 4: Area of the Zoo Negara

Bird house

Children's World



Aviary

Lake

Breading area

Animal Show

Figure 5: Areas of the sample collection

4.2 Specimen collection

4.2.1 Samples from birds

A total of 116 feces specimen from 71 species of birds were collected and kept in the fecal containers labeled according to the bird species and location. Fecal sample of birds were collected carefully to avoid any contamination with soil and other contaminants. Collection of fecal specimens and the identification of bird species from their respective locations were carried out in the morning with the assistance of the bird handlers. The specimens were brought back to the Department of Parasitology, Faculty of Medicine, University of Malaya and stored in the cold room at 4°C before processing.

4.2.2 Samples from bird handlers

Sixteen fecal containers were distributed to the bird handlers for fecal collection but only eight containers were returned. The returned samples came from the bird handlers who are working at birdhouse and children's world. The profile of the bird handlers such as names, age, race, gender and the location of work were recorded. All samples were brought back to the laboratory to be processed.

4.3 Stool examination for *Cryptosporidium* oocyst

4.3.1 Preparation of faecal smears

Direct smear and formalin ethyl acetate concentration technique were employed for all stool samples from birds and bird handlers. The number of C*ryptosporidium* oocyst on the direct smear may be low. Therefore, concentrating the sample would increase the probability of detecting and identifying the present of *Cryptosporidium* oocysts. In this study, formalin ethyl acetate concentration technique was utilized. All materials, reagents and also the procedure for formalin ethyl acetate concentration technique are listed in Appendix A.

4.3.2 Staining process

The faecal smears were stained using the Ziehl Neelson (acid-fast) stain. The materials and methods of this staining technique are listed in Appendix B. After staining, the slides were examined under 400x. Putative *Cryptosporidium sp.* oocysts appeared as bright rose-pink spheres (5 ± 1 μm) on a bluish green background. The positive slides were examined again under 1000x magnification to confirm the presence of *Cryptosporidium* oocyst. All slides positive for *Cryptosporidium sp.* oocysts using the Ziehl Neelson staining method were recorded.

CHAPTER 5: RESULTS

5.0 RESULTS

A total of 116 fecal specimens from birds and 8 fecal specimens from bird handlers were collected from the Kuala Lumpur National Zoo (Table 2 and Table 4). All specimens from birds were collected according to their locations (Table 3). By using the Ziehl-Neelson staining technique, 4 specimens (3.4%) from birds (Table 2) and one sample from a bird handler were positive (Table 4). *Cryptosporidium* oocysts were identified as bright rose pink spheres with the size of 5 ± 1 µm on a bluish green background (Figure 6a and 6b).

The four positive samples for *Cryptosporidium* oocyst in birds were detected from the Great Currasow (*Crax rubra*) and Wreathed Hornbill (*Aceros undulates*) (both located at the aviary), Common Peafowl (*Pavo cristatus*) (located at the lake) and Bushy-crested Hornbill (*Anorrhinus galeritus*) (located at the birdhouse) (Table 2) (Figure 7). Only 14.3% (1 out of 7) of the specimens taken from the Common Peafowl, 50% (1 out of 2) of the specimens taken from the Wreathed Hornbill, and 100% (1 out of 1) of the specimen from Bushy-Crested Hornbill and Great Currasow were positive for *Cryptosporidium* oocysts. Specimens from the bird handlers showed 12.5% (1 out of 8) was positive with *Cryptosporidium* oocysts (Table 4). Locations that were free from *Cryptosporidium* oocysts were Children's World, Animal Show and the Breeding Area. Birds at the lake showed the highest percentage (12.5%) of being positive followed by birds at the aviary (5.4%) and birdhouse (2.8%) (Table 3).

TABLE 2: List of birds and specimens positive for *Cryptosporidium* oocysts using Ziehl-Neelson staining technique

No	Common Name	Species	Location	No. of specimens taken	No. positive for *Cryptosporidium* oocyst
1	African Grey Parrot	*Psittacus erithacus*	BH	1	-
2	African Ground Hornbill	*Bucorvus leadbeateri*	BH	1	-
3	African Spoonbill	*Platalea alba*	A	1	-
4	All domestic chicken	*Gallus gallus*	CW	2	-
5	Barn Owl	*Tyto alba*	BH,AS	2	-
6	Barred Eagle-Owl	*Bubo sumatranus*	CW,AS,BH	3	-
7	Black Hornbill	*Anthracoceros malayanus*	BH	1	-
8	Black kite	*Mulvus migrans*	BH	1	-
9	Black Swan	*Cygnus atratus*	L	2	-
10	Blue and yellow macow	*Ara ararauna*	BH,AS,CW	3	-
11	Blue-breasted Quail	*Coturnix chinensis*	BH	1	-
12	Blyth's Hawk Eagle	*Spizaeus alboniger*	BH	1	-
13	Brahminy Kite	*Haliastur Indus*	BH,AS	3	-
14	Brown barbet	*Calorhamphus fuliginosus*	BH	1	-
15	Buffty Fish Owl	*Ketupa ketupu*	BH	1	-
16	Bulbul	*Pyenonotus*	CW	1	-
17	Bushy-crested Hornbill	*Anorrhinus galeritus*	BH	1	1
18	California Quail	*Callipela californica*	BH	1	-
19	Cassowary		BH	1	-
20	Changeable Hawk Eagle	*Spizaeus cirrhatus*	AS	1	-
21	Common Peafowl	*Pavo cristatus*	L,AS,CW,	6	1
22	Crested Fireback Pheasant	*Lophura ignita*	BA,BH,A	3	-

23	Crested serpent Eagle	*Spilornis cheela*	BA	1	-
24	Crestless Fireback Pheasant	*Lophara erythrophthalma*	BA,BH,A	3	-
25	Domestic Duck	*Anas domesticus*	A	1	-
26	Eclectus Parrot	*Eclectus roratus*	AS	1	-
27	Egyptian Goose	*Alopochen aegyptiacus*	L	1	-
28	Emu	*Dromalus novaehollandiea*	A	1	-
29	Golden Phesant	*Chrysolophus pictus*	A	1	-
30	Great Argus Pheasant	*Argusianus argus*	A,BH,BA	3	-
31	Great Currasow	*Crax rubra*	A	1	1
32	Greater Hornbill	*Bucerros bicornis*	BH,AS,A	6	-
33	Greater sulfur Crested Cockatoo	*Cacatua galerita*	BH	1	-
34	Green jungle Fowl	*Gallus varius*	BH	1	-
35	Green-Winged Dove	*Chalcophaps indica*	A	1	-
36	Green-winged macaw	*Ara chloroplera*	AS	1	-
37	Helmeted Guineafowl	*Numida meleagris*	A,CW	2	-
38	Hill Myna	*Gracula religiosa*	BH	1	-
39	Java sparrow	*Padda oryzivora*	CW	1	-
40	Lesser Fish Eagle	*Ichthyophaga humilis*	BH	1	-
41	Lesser Sulphur Crested Cockatoo	*Cacatua sulphurea*	CW	1	-
42	Little Corella	*Cacatua sanguinea*	CW	1	-
43	Lovebird	*Agopornis fisheri*	CW,BH,A	4	-
44	Magpie Robin	*Copsychus saularis*	BH	1	-

45	Malayan Peacock-Pheasant	*Polyplectron malacense*	BH,BA	2	-
46	Mandarin Duck	*Aix galericulata*	A	1	-
47	Milky Stork	*Mycteria leucocephala*	A	1	-
48	Moluccan cockatoo/ salmon-crested cockatoo	*Cacatua moluecencis*	AS	1	-
49	Mountain Peacock-Pheasant	*Polyplectron inopination*	BH	1	-
50	Nicobar Pigeon	*Caloenas nicobarica*	A	1	-
51	Oriented Pied Hornbill	*Anthracoceros albirostris*	BH	1	-
52	Ostrich	*Struthio camelus*	Savanna	2	-
53	Painted Stork	*Mycteria cinerea*	L,BH	2	-
54	Pigeon	*Columba*	CW,AS	3	-
55	Pin-tailed Parrot Finch	*Erythuraprasima*	BH	1	-
56	Pink-backed Pelican	*Pelecanus rufescens*	A	1	-
57	Rainbow Lory	*Trichoglossus haematodus*	CW	1	-
58	Red Junglefowl	*Gallus gallus*	A	1	-
59	Rhinoceros Hornbill	*Buceros rhinoceros*	BH,A	3	-
60	Scarlet Macow	*Ara macoa*	CW	1	-
61	Spotted billed pelican	*Pelecanus rufescens*	L	1	-
62	Silver Pheasant	*Lophura nycthemera*	A,BH,BA	4	-
63	Spotted wood-owl	*Strix seloputo*	BH,AS	2	-
64	Storm's Stork	*Ciconia stormi*	L	2	-
65	Swan Goose	*Anser cygnoides*	L	2	-
66	Turkey	*Meleagris gallopavo*	CW	2	-
67	White Bellied Sea Eagle	*Haliaetus leucogaster*	BH,AS	2	-
68	White Cockcatoo	*Cacatua alba*	BH	1	-

69	White rumped Shama	*Copsychus malabaricus*	BH	1	-
70	Wreathed Hornbill	*Aceros undulates*	A,BH	2	1
71	Wrinkled Hornbill	*Aceros corrugatus*	A	1	-
		Total		116	4

A = Aviary

AS = Animal show

BA = breeding area

BH = Birdhouse

CW = Children's world

L = Lake

TABLE 3: Percentage of fecal bird specimens positive for *Cryptosporidium* oocysts according to location

No.	Location	Total no of fecal specimens collected	No. of specimens positive for *Cryptosporidium*	Percentage positive
1	Animal Show	13	-	0
2	Aviary	37	2	5.4
3	Bird House	35	1	2.8
4	Breeding area	5	-	0
5	Children's World	18	-	0
6	Lake	8	1	12.5

TABLE 4: Biodata of the bird handlers examined for *Cryptosporidium* oocysts

No.	Name	Age (years)	Gender	Race	Location	Result (presence of *cryptosporidium* oocysts)
1	J	26	M	Malay	Bird house	negative
2	S	27	F	Malay	Bird house	negative
3	M K	21	M	Malay	Bird House	**positive**
4	M E	25	M	Malay	Bird House	negative
5	Z	19	M	Malay	Bird House	negative
6	N B H	20	F	Malay	Children's world	negative
7	M Y	52	M	Malay	Children's world	negative
8	M A M S	20	M	Malay	Children's world	negative

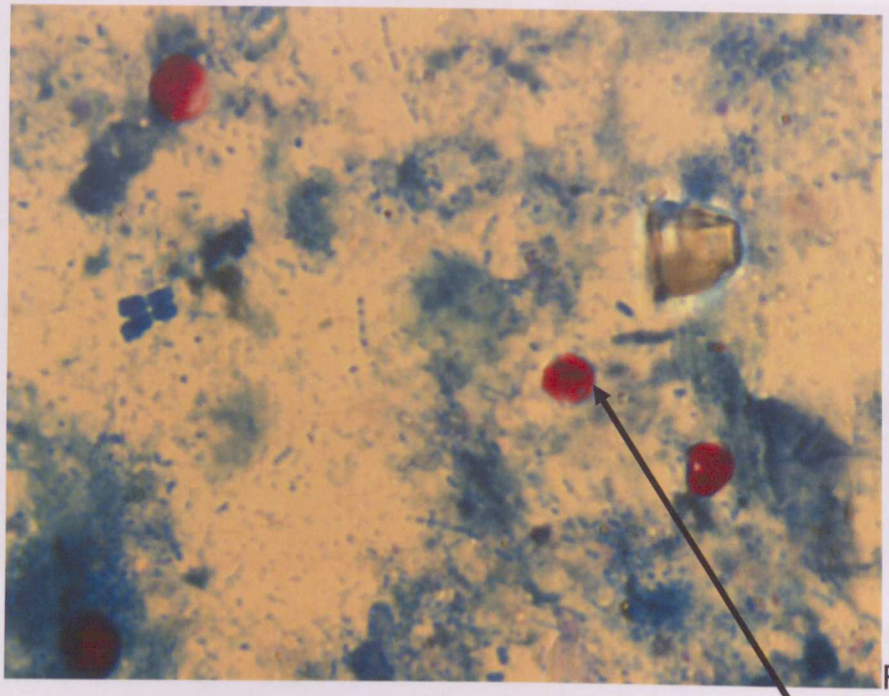


Figure 6(a)

Cryptosporidium oocyst

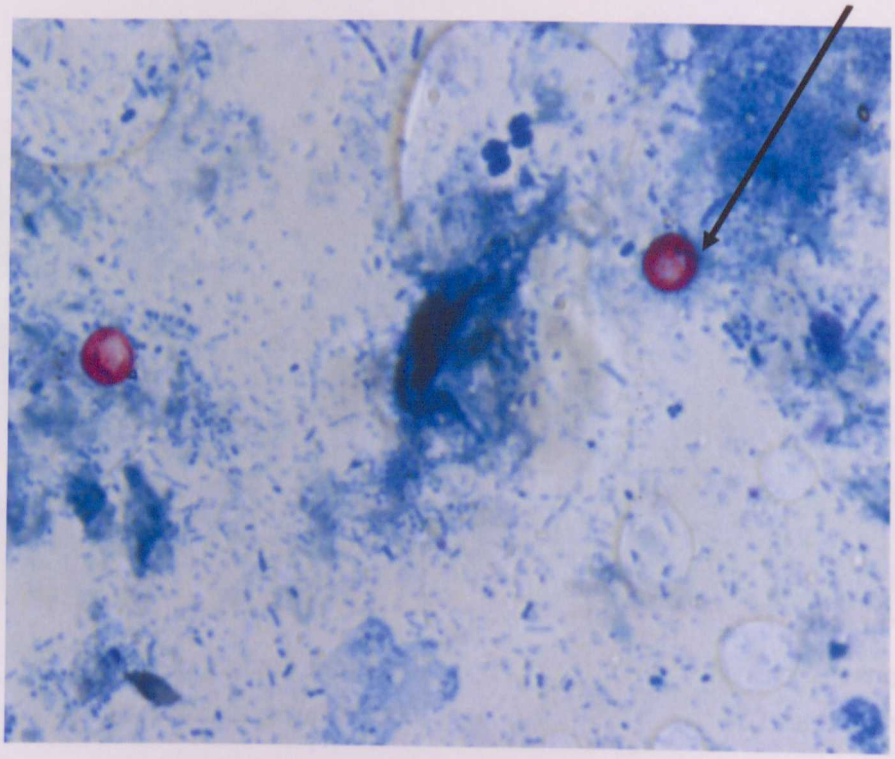
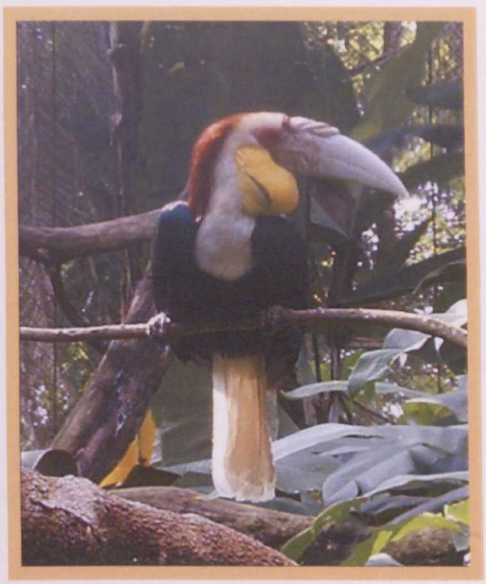


Figure 6(b)

Figure 6(a)(b): *Cryptosporidium* oocysts that are found under microscopy examination (1000X magnification)

Common Peafowl
(*Pavo cristatus*)



Wreathed Hornbill
(*Aceros undulates*)

Bushy-crested Hornbill
(*Anorrhinus galeritus*)

Great Currasow
(*Crax rubra*)

Figure 7: Birds positive with *Cryptosporidium* oocysts

CHAPTER 6: DISCUSSION

6.0 DISCUSSION

Cryptosporidium is a ubiquitous enteric protozoan pathogen that can infect many types of animals including human. Animals from the group of reptiles, mammals, birds, and fishes can be affected with cryptosporidiosis. Studies have confirmed the presence of *Cryptosporidium* oocysts in pets, domestic and wild animals (Sterling and Arrowood, 1978; O'Donoghue, 1995; Lim and Ahmad, 2001). The oocysts of *Cryptosporidium spp.* even can be found in the animals such as dugong (*Dugong dugon)* (Morgan, 2000), lizard and snake (Zhou *et al,* 2003).

Since the first detection of *Cryptosporidium* in birds in 1929 by Tyzzer (Sterling and Arrowood, 1978; Current, 1989), many studies have been carried out to determine the presence of *Cryptosporidium* oocysts in various species of birds. However, there are very few studies which have ascertain the naturally occurring cryptosporidiosis in wild birds. Prevalence rate of 5.9% to 27.3% have been demonstrated in flocks in the United States (Lindsay and Blagburn, 1990).

Previous study carried out in 2004 at Kuala Lumpur National Zoo showed that 6 species of birds were positive with *Cryptosporidium* oocyst in their feces. The positive species included Wrinkled Hornbill (*Aceros corrugatus*), Great Argus Pheasant (*Argusianus argus*), Black Swan (*Cygnus atratus*), Swan Goose (*Anser cygnoides*), Marabou Stork (*Leptoptilos crumeniferus*) and Moluccan Cockatoo (*Cacatua moluccencis*). These birds

were located in the aviary and lake and the Moluccan Cockatoo was routinely used as a show bird (Rohela *et al.* 2005).

This present study showed a prevalence of 3.4% (4 out of 116) of *Cryptosporidium spp.* in bird's feces. The prevalence rate has decreased compared to the previous study (6%) which was conducted 2 years ago at the same sites. In this study, *Cryptosporidium* oocysts were detected in the feces of the Wreathed Hornbill (*Aceros undulates*) and Great Currasow (*Crax rubra)* from the aviary, Bushy-crested Hornbill (*Anorrhinus galeritus*) from the bird house and Common Peafowl (*Pavo cristatus)* from the lake (Table 5).

Although the prevalence of *Cryptosporidium spp.* in birds at the Kuala Lumpur National Zoo has decreased to 3.4% compared to the previous study, the birds recorded positive in this study were different from the ones that were positive in the previous study. All 4 types of birds that were positive with *Cryptosporidium* oocysts were not found to be positive before. This finding indicates that *Cryptosporidium* infection might be spreading to other species of birds in the zoo. In both the previous and present studies, birds that were found to be positive for *Cryptosporidium* were all asymptomatic. It is very likely that these birds were not quarantined or separated from the other birds that do not harbour the parasite because they did not present any symptoms therefore facilitating the transmission of *Cryptosporidium* oocysts to the other birds.

There are only three species of *Cryptosporidium* that can infect birds. They are *C. meleagridis, C. galli, and C. baileyi*. Most studies of cryptosporidiosis in birds discovered birds with *C. baileyi* (Lindsay and Blagburn, 1990). Cryptosporidial infections in birds occur in the conjunctiva, nasopharynx, larynx, trachea, bronchi, air sacs, proventriculus, small intestine, large intestine, ceca, cloaca, Bursa of Fabricius, kidneys and urinary tract. Cryptosporidiosis in birds may be a respiratory, enteric and renal disease. Which symptoms are present, or in rare cases which combinations of symptoms, depends in part on which species of birds are infected and which species of *Cryptosporidium* is causing the infection (Sterling and Arrowood, 1978).

Table 5: Names and locations of the positive sample

No.	Species of birds	Location
1	Wreathed Hornbill (*Aceros undulates*)	Aviary
2	Great Currasow (*Crax rubra*	Aviary
3	Bushy-crested Hornbill (*Anorrhinus galeritus*)	BirdHouse
4	Common Peafowl (*Pavo cristatus)*	Lake

In terms of location, a comparison between the two studies showed that the aviary and lake were still contaminated with *Cryptosporidium* oocysts whereas location that was positive in 2004 which was Animal Show was negative for *Cryptosporidium* in this present study. On the other hand, the location that was previously negative in 2004 which was birdhouse was found to be positive in this present study.

Although there were three locations (eg. aviary, birdhouse and lake) in this present study that were positive for cryptosporidiosis, the probability of birds acquiring cryptosporidiosis might be higher in the lake because birds could get infected not only via contaminated food or close contact but also through contaminated water as well. This postulation is confirmed with the findings of this study whereby birds at the lake showed the highest percentage of positive samples for *Cryptosporidium* oocysts (12.5%) followed by the birds in the aviary (5.4%) and (2.8%) at the bird house. Birds at the Animal Show, breeding area and Children's World were found to be negative for *Cryptosporidium* oocysts.

There are a few contributing factors to the high percentage of prevalence of avian cryptosporidiosis in the lake. Birds at the lake are not caged therefore they are free to mix with other birds or fly to any location. They might fly out of the zoo compound. This will facilitate the transmission of *Cryptosporidium* to other areas inside or outside the zoo compound. The implication of birds in the lake being infected is great simply because the lake

water may be contaminated with *Cryptosporidium* oocysts-laden feces and this could be a source of transmission to other birds, animals and human who come in contact with the contaminated water. Many studies and waterborne outbreaks have implicated contaminated water as a source of infection of cryptosporidiosis (Xiao *et al,* 2004; Current, 1989; O'Donoghue, 1995). One of the biggest waterborne outbreaks was recorded in 1993 at Milwaukee, Wisconsin, USA. An estimated 1.5 million people were exposed to C*ryptosporidium* contamination in the public water supply. Out of this total number, 403,000 became ill and 104 people died (mostly were immunocompromized) (MacKenzie *et al*, 1994).

Occurrence of *Cryptosporidium* oocysts in the aviary is probably due to cross contamination. This is because the birds at the aviary are always being relocated from one cage to another. As an example, an asymptomatic infected bird from cage A might be transferred to cage B. Therefore if cage A is not washed properly it might still be contaminated with the feces of the previously infected bird that was transferred. When a new free of infection bird is placed in cage A, there is a chance for it to acquire the infection via the contaminated feces. This scenario enables the transmission of the *Cryptosporidium* oocysts to other birds if it is not controlled properly.

This study has discovered that one of the bird handlers who were stationed at the bird house was infected with cryptosporidiosis. This person does not only manage the birds in the bird house but he also manages the

birds at the aviary and lake. As indicated earlier, all these locations that this bird handler is working were positive with infected birds. Therefore it is difficult to conclude where he acquired the infection from or whether he was the source of infection to the birds. This is because of the three avian *Cryptosporidium* sp. (eg. *C. meleagridis, C. baleyi and C galli*), only *C. meleagridis* can infect both humans and birds (Xiao *et al*, 2004). Only with the employment of molecular techniques can this dilemma be determined through speciation or genotyping.

The common clinical feature of cryptosporidiosis in immunocompetent and immunocompromised persons is diarrhea; it is this symptom that most often leads to diagnosis. Characteristically, the diarrhea is profuse and watery, it may contain mucus but rarely blood and leucocytes, and it is often associated with weight loss. Other less common clinical features include abdominal pain, nausea and vomiting, and low-grade fever (<39°C). Occasionally, non-specific symptoms such as myalgia, weakness, malaise, headache and anorexia occur. Severity of these symptoms may wax and wane in individuals and usually parallels the intensity of oocyst shedding. Both the duration of symptoms and the outcome typically vary according to the immune status of the host. AIDS patients usually experience a prolonged, life threatening illness, whereas most immunocompetent persons experience a short –term illness with complete, spontaneous recovery. (Current, 1989).

However, the clinical presentation of gastrointestinal cryptosporidiosis does not always fit within one of these two divergent categories. Person with clinical and laboratory features of AIDS have been reported to clear infections after several months of diarrhea, and individuals reported to be immunocompetent have had infections lasting more than one month (Current, 1986). In immunocormpromise host, diarrheas illness due to *Cryptosporidium* infection of the gastrointestinal tract becomes progressively worse with time and may be a major factor leading to death. It is believed that the infection usually begins with organisms colonizing the ileum and jejunum and develops into a life-threatening condition when a large portion of the gastrointestinal mucosa is covered with what has been described as a monolayer of parasites. Fluid loss in patients with AIDS and cryptosporidiosis is often excessive; 3-6 liters of diarrheic stool per day is common, and as much as 17 liters of watery stool per day has been reported.

Many studies had showed that the animal handlers can easily get infection from the animals they handle. A report showed that 11 samples out of 16 were positive with *Cryptosporidium* oocysts from a person who has direct contact with calves (Anonymous, 1982). In this present study, the bird handlers may have gotten *Cryptosporidium* oocysts during their direct contact with the infected birds. Bad personal hygiene is one of the factors that may have caused the bird handlers to acquire the infection. It will be paramount then to establish in future studies the details of the association of human and avian cryptosporidiosis.

6.1 Recommendations for future study

It is suggested that for future study, the quality of water from the lake is to be looked into in order to determine the association of waterborne cryptosporidiosis in a zoological setup. The water samples from the lake should be collected from different parts of the lake.

As mentioned earlier in the discussion, it is a difficult task to identify the species of the *Cryptosporidium* via microscopy. The only way to identify the species is by employing molecular technique such as Polymerase Chain Reaction (PCR). Molecular biology has provided powerful new tools for characterizing *Cryptosporidium*. Molecular tools have enabled not only the identification of species and genotypes in the faeces of infected hosts but also their recognition in environmental samples, including water. Seven species which are *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. suis* and *C. muris* are associated with human disease, and molecular approaches have enabled a greater understanding of the contributions of humans and livestock as reservoirs of *Cryptosporidium* infection.

CHAPTER 7: CONCLUSION

7.0 CONCLUSION

A comparison between a study in 2004 and the present study conducted on the birds at National Zoo, Kuala Lumpur showed there was a decrease in the prevalence rate in the birds. However there seemed to be an indication that cryptosporidiosis might be spreading to other species of birds and to other locations in the zoo which were not previously documented. This study also discovered the probable association of cryptosporidiosis among birds and their bird handlers. However, conclusive remarks can only be done after the confirmation of species found in birds and bird handlers through molecular identification.

CHAPTER 8: APPENDIX

8.0 APPENDIX

APPENDIX A:

Formalin ethyl acetate concentration technique

Materials:-

i) Applicator sticks

ii) 15ml glass centrifuge tubes

iii) Centrifuge machine

iv) Sieve

v) Glass beaker

vi) Glass slides

vii) Cover slides

viii) 10% formal saline. (10% formalin in normal saline)

ix) Ethyl acetate

Method:-

About 10 ml of formal-saline was filled into a clean centrifuge tube.

↓

About 1 gram of feces (size of a pea) was placed into the centrifuge tube.

↓

Content was emulsified

↓

2.5 ml of ethyl acetate was added into the centrifuge tube.

↓

It was shaken well for about 30 seconds.

↓

The content inside the centrifuge tube was poured through a sieve into a glass beaker.

↓

The filtrate was poured back into a clean centrifuge tube.

↓

The tube was centrifuge for 30 second at 500rpm,
30 seconds at 1000rpm,
30 seconds at 1500rpm and
30 seconds at 2000rpm.

↓

The supernatant was poured out and sediment was collected.
A few drops of sediment were placed on the glass slide to make a smear.

↓

Smear was dried

APPENDIX B:

Modified Ziehl Neelson staining technique

Materials:

i) Absolute methanol

ii) Cold Strong Carbol Fuchsin

iii) 3% acid alcohol

iv) Methylene Blue

v) Coplin jar

- 3% acid alcohol prepared from:
  - 3ml of HCL
  - 97ml of 95% ethanol

Method:

A smear was made on a glass slide and allowed to dry

↓

The smear was fixed with methanol for about 5 minutes

↓

The smear was flooded with cold strong carbol fuchsin and was left for about 5 to 10 minutes.

↓

It was then rinsed with tap water

↓

It was differentiated with acid alcohol about 30 seconds

(Until the red colour flood out)

↓

It was rinsed again

↓

The smear was caunter stained with methylene blue for about 30 seconds.

↓

It was rinse with tap water

↓

Sections was blotted dry

↓

Slide were examined using light microscope

CHAPTER 9: REFERENCES

9.0 REFERENCES

Anonymous. Human cryptosporidiosis – Alabama. Center for disease control and prevention. MMWR Morb Mortal Wkly Rep. 1982 May 21;31(19):252-4

Alves M, Xiou L, Antunes F, Matos O. Distribution of *Cryptosporidium* subtype in humans and domestic and wild rumments in Portugal. Parasitol Res 2006; 10: 164-9.

Augus KW. Cryptosporidiosis in men, domestic animals and birds: a review. Journal of Royal Society of Medicine 1983; 76: 62-70.

Awad-el-Kariem FM, Warhurst DC, McDonald V. Detection and species identification of *Cryptosporidium* oocysts using a system based on PCR and endonuclease restriction. *International Journal for Parasitology* 1997; 27: 1531-1536.

Current WL. Cryptosporidiosis. In: McAdam KPWJ, editor. New Strategies in Parasitology. London: Glaxo Group Research Limited; 1989. p. 257-75

Current WL. *Cryptosporidium:* its biology and potential for environmental transmission. CRC Crit Rev Envir Control 1986; 17: 21-31

Dhillon AS, Thacker HL, Dietzel AV, Winterfield RW. Respiratory cryptosporidiosis in broiler chickens. *Parasitology* 1994; 109: 19-22.

Fayer R. *Cryptosporidium* : a water-borne zoonotic parasite. Veterinary Parasitology 2004; 62: 37-56

Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium* : transmission, detection and identification. International journal of Parasitology 2000; 1305-22

Graczyk TK, Cranfield MR, Fayer R. Viability and infectivity of *Cryptosporidium parvum* oocysts are retained upon intestinal passage through a refractory avian host. Applied and Environmental Microbiology 1996: 3234-7

Hoerr FJ, Current WL, Haynes TB. Fatal cryptosporidiosis in quail. *Avian Diseases* 1986; 30: 421-425.

Lim YAL, Ahmad RA. Occurrence of *Giardia* and *Cryptosporidium sp.* in rodents in the vicinity of the Temuan Orang Asli community. Malaysian Appl Biol 2001; 30: 39-45.

Lindsay DS, Blagburn BL. Cryptosporidiosis in birds. In: Dubey JP, Speer CA, Fayer R, editor. Cryptosporidiosis of man and animals. Boca Raton: CRC Press; 1990. p. 125-48

Lionel SL, Prathibha V, Peter G, Larry E. Cryptosporidiosis Outbreak in a Recreational Swimming Pool in Minnesota. Journal of Environmental Health 2004; 67:16

MacKenzie WR, Hoxie NJ, Proctor ME, et al. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N England J Med 1994; 331: 161-7

Meisel JL, Perera DR, Meliagro C, Rubin CE. Overwhelming watery diarrea associated with a *Cryptosporidium* in an immunosuppressed patient. Gastroenterology 1976; 70: 1156-60

Morgan UM, Monis PT, Xiao L, Limor J, Sulaiman I. Molecular and phylogenetic characteristic of *Cryptosporidium* from bird. International Journal for Parasitology 2001; 31(3) : 289-96

Morgan UM, Xiao L, Hill BD, O'Donoghue P *et al.* Detection of the *Cryptosporidium parvum* "human" genotype in a dugong (Dugong dugon). J Parasitol. 2000; 86(6):1352-4

Nime FA, Burek JD, Page DL, Holscher MA, Yardley JH. Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium.* Gastroenterology 1976; 70: 592-8

O'Donoghue PJ. *Cryptosporidium* and Cryptosporidiosis in man and animals. International journal for Parasitology 1995; 25(2): 139-95

Rohela M, Lim YAL, Khadijah PYY, Laang ST, Nazri MHM, Nurulhuda Z. Occurrence of *Cryptosporidium* oocysts in Wrinkled Hornbill and other birds in The Kuala Lumpur National Zoo. Southeast Asian J Trop Med Public Health 2005; 36(4): 34-40.

Slavin D. *Cryptosporidium meleagridis* (sp. nov.). *Journal of Comparative Pathology (and Therapeutics)* 1955; 65: 262-266.

Soave R and Armstrong D. *Cryptosporidium* and cryptosporidiosis. Reviews of Infection Diseases 1986; 8 (6): 1012-23

Sterling CR, Arrowood MJ. Cryptosporidia. In: Kreter JP, editor. Parasitic Protozoa. 6. London: Academic Press Limited; 1978. p. 159-225.

Sréter T, Varga I. Cryptosporidiosis in birds — A review. *Veterinary Parasitology* 1999; 87: 261-279.

Walter Q, Betancourt, Joan BR. Drinking water treatment processes for removal of *Cryptosporidium* and *Giardia*. Veterinary parasitology 2004; 126: 219-34

Weber J. Sexually acquired parasitic infections in homosexual men. Parasitology Today 1985; 1 (4): 93-5

Xiao L, Fayer R, Ryan U, Upton SJ. *Cryptosporidium* Taxonomy: recent advances and implications for public health. Clin Microbiol Rev 2004; 17: 72-79

Zhou L, Yang C, Xiao L. PCR-mediated recombination between *Cryptosporidium spp.* of lizards and snakes. J Eukaryot Microbiol. 2003; 50: 563-5

CHAPTER 10: EXTENDED ABSTRACT

10.0 EXTENDED ABSTRACT

INTRODUCTION

Cryptosporidium is a ubiquitous enteric protozoan pathogen that infects humans, domestic and feral animals, worldwide. It is an important causative agent of diarrhoeal disease in man, leading to significant morbidity and mortality in both developing and developed countries. Cryptosporidiosis, a disease caused by *Cryptosporidium,* was first described in the caeca of chickens by Tyzzer in 1929. Subsequently, a report in 1955 described structurally similar parasites in turkeys and these parasites were later named *C. meleagridis* (Slavin, 1955). In 1986, Current, Upton and Haynes isolated and described an organism from chickens and gave the name *C. baileyi* to the species.

Currently, there are 16 species of *Cryptosporidium* which have been identified having different morphologies and hosts (Xiao *et al*, 2004). *Cryptosporidium* species that have been described in birds are *C. meleagridis*, *C. baileyi* and *C. galli* (Awad-el-Kariem *et al*, 1997). Only *C. meleagridis*, which infects turkeys and parrot is a known threat to human beings. Nevertheless, *C. baileyi* is probably the most common avian *Cryptosporidium* species since it is able to infect chickens, turkeys, ducks, cockatiels, quails and ostriches whereas *C.galli*, the latest addition to the family infects hosts such as finches, domestic chickens, capercaille and pine grosbeaks (Xiao *et al,* 2004).

In birds, *Cryptosporidium sp.* has not only been reported in chickens (Sterling and Arrowood, 1978), turkeys (Srèter and Varga, 1999), quails, pheasants, peafowls, junglefowls, ducks, geese, parrots, pinches, lovebirds, budgerigars, ostriches (Morgan *et al*, 2001), catercaille, pine grosbeaks (Xiao *et al*, 2004), but the latest addition to this list is the wrinkled hornbill found in Malaysia (Rohela *et al*, 2005). Avian cryptosporidiosis can manifest itself as respiratory (Dhillon *et al.* 1994) and intestinal (Lindsay *et al*, 1990) diseases. In some cases, cryptosporidiosis might even manifest as renal disease and may be fatal (Hoerr *et al,* 1986).

The association between cryptosporidiosis in animals and animal handlers is a topic of interest. In 1981, a case of cryptosporidiosis in an animal handler was reported. The animal handler was a previously healthy 25 year old male who had symptoms of cryptosporidiosis such as nausea, low-grade fever, moderate abdominal cramps, anorexia, 5 to 10 watery, frothy bowel movements a day and constipation. Diagnosis was confirmed with the finding of *Cryptosporidium sp.* oocysts in the feces (Anonymous, 1982). Following this report, many similar cases of cryptosporidiosis were reported among animal handlers (Augus, 1983; Graczyk, 1996).

In 2004, a study on the occurrence of *Cryptosporidium sp.* oocysts in the fecal samples of birds in Zoo Negara, Kuala Lumpur was conducted. The study showed 6 species of birds were infected with *Cryptosporidium* (Rohela *et al,* 2005). This present study is an expansion of the previous study. This

study tries to ascertain not only the current prevalence rate in the birds but also to determine the association between cryptosporidiosis in birds and the bird handlers.

OBJECTIVES

The main objectives of this study are:-

1. To re-evaluate the occurrence of *Cryptosporidium sp.* oocyst in the fecal samples of birds in the Zoo Negara, Kuala Lumpur, Malaysia.
2. To determine the prevalence of cryptosporidiosis among bird handlers and its association with the occurrence of cryptosporidiosis in birds.

METHODOLOGY

LOCATION OF STUDY

The study was carried out in Kuala Lumpur National Zoo, located at Ulu Kelang, 13 km northeast of Kuala Lumpur. The birds at the National Zoo can be found at various places such as the aviary, bird house, breeding area, animal show, children's world and lake. All birds in the locations mentioned are caged except those in the lake. Birds housed in the aviary are birds of various species which include smaller birds such as Lovebirds to the larger birds such as Common Peafowl and Hornbill. Birdhouse and breeding area are places where the birds are quarantine before being sent to the aviary for visitors to view. It is also a place for the offspring of the birds. Children's world is designed especially for children to have a closer view of domesticated animals and birds such as macaw and parrot. Birds used at the animal show

are trained to perform stunts and entertaining activities. Storks and pelicans are the types of birds commonly found in the lake where they are free to roam.

SAMPLE COLLECTION

A total of 116 fecal specimens from 71 species of birds were collected and kept in the fecal containers labeled according to the bird species and location. Fecal sample of birds were collected carefully to avoid any contamination with soil and other contaminants. Collection of fecal specimens and the identification of bird species from their respective locations were carried out in the morning with the assistance of the bird handlers. The specimens were brought back to the Department of Parasitology, Faculty of Medicine, University of Malaya and stored in the cold room at 4°C before processing.

Sixteen fecal containers were distributed to the bird handlers for fecal collection but only eight containers were returned. The profile of the bird handlers such as names, age, race, gender and the location of work were recorded. All samples were brought back to the laboratory to be processed.

STOOL EXAMINATION FOR *CRYPTOSPORIDIUM* OOCYSTS

Direct smear and formalin ethyl acetate concentration technique were employed for all stool samples from birds and bird handlers. The smears were stained using the Ziehl Neelson (acid-fast) stain. After staining, the slides were examined under 400x. Putative *Cryptosporidium sp.* oocysts appeared

as bright rose-pink spheres (5 ± 1 µm) on a bluish green background. The positive slides were examined again under 1000x magnification to confirm the presence of *Cryptosporidium* oocyst. All slides positive for *Cryptosporidium sp.* oocysts using the Ziehl Neelson staining method were recorded.

RESULTS

A total of 116 fecal specimens from birds and 8 fecal specimens from bird handlers were collected from the Kuala Lumpur National Zoo (Table 2 and Table 4). All specimens were collected according to their locations (Table 3). By using the Ziehl-Neelson staining technique, 4 specimens (3.4%) from birds (Table 2) and one sample from a bird handler were positive (Table 4). *Cryptosporidium* oocysts were identified as bright rose pink spheres with the size of 5 ± 1 µm on a bluish green background.

The four positive samples for *Cryptosporidium* oocyst in birds were detected from the Great currasow (*Crax rubra*) and Wreathed Hornbill (*Aceros undulates*) (both located at the aviary), Common peafowl (*Pavo cristatus*) (located at the lake) and Bushy-crested Hornbill (*Anorrhinus galeritus*) (located at the birdhouse) (Table 2). Only 14.3% (1 out of 7) of the specimens taken from the Common Peafowl, 50% (1 out of 2) of the specimens taken from the Wreathed Hornbill, and 100% (1 out of 1) of the specimen from Bushy-Crested Hornbill and Great Currasow were positive for *Cryptosporidium* oocysts. Specimens from the bird handlers showed 12.5% (1 out of 8) was positive with *Cryptosporidium* oocysts (Table 4). Locations that were free from *Cryptosporidium* oocysts were children's world, animal show

and the breeding area. Birds at the lake showed the highest percentage (12.5%) of being positive followed by birds at the aviary (5.4%) and birdhouse (2.8%) (Table 3).

TABLE 2: List of birds and specimens positive for *Cryptosporidium* oocysts using Ziehl-Neelson staining technique

No	Common Name	Species	Location	No. of specimens taken	No. positive for *Cryptosporidium* oocyst
1	African Grey Parrot	*Psittacus erithacus*	BH	1	-
2	African Ground Hornbill	*Bucorvus leadbeateri*	BH	1	-
3	African Spoonbill	*Platalea alba*	A	1	-
4	All domestic chicken	*Gallus gallus*	CW	2	-
5	Barn Owl	*Tyto alba*	BH,AS	2	-
6	Barred Eagle-Owl	*Bubo sumatranus*	CW,AS,BH	3	-
7	Black Hornbill	*Anthracoceros malayanus*	BH	1	-
8	Black kite	*Mulvus migrans*	BH	1	-
9	Black Swan	*Cygnus atratus*	L	2	-
10	Blue and yellow macow	*Ara ararauna*	BH,AS,CW	3	-
11	Blue-breasted Quail	*Coturnix chinensis*	BH	1	-
12	Blyth's Hawk Eagle	*Spizaeus alboniger*	BH	1	-
13	Brahminy Kite	*Haliastur Indus*	BH,AS	3	-
14	Brown barbet	*Calorhamphus fuliginosus*	BH	1	-
15	Buffty Fish Owl	*Ketupa ketupu*	BH	1	-
16	Bulbul	*Pyenonotus*	CW	1	-
17	Bushy-crested Hornbill	*Anorrhinus galeritus*	BH	1	1
18	California Quail	*Callipela californica*	BH	1	-
19	Cassowary		BH	1	-
20	Changeable Hawk Eagle	*Spizaeus cirrhatus*	AS	1	-
21	Common Peafowl	*Pavo cristatus*	L,AS,CW,	6	1
22	Crested Fireback Pheasant	*Lophura ignita*	BA,BH,A	3	-
23	Crested serpent Eagle	*Spilornis cheela*	BA	1	-
24	Crestless Fireback Pheasant	*Lophara erythrophthalma*	BA,BH,A	3	-
25	Domestic Duck	*Anas domesticus*	A	1	-
26	Eclectus Parrot	*Eclectus roratus*	AS	1	-

27	Egyptian Goose	*Alopochen aegyptiacus*	L	1	-
28	Emu	*Dromalus novaehollandiea*	A	1	-
29	Golden Phesant	*Chrysolophus pictus*	A	1	-
30	Great Argus Pheasant	*Argusianus argus*	A,BH,BA	3	-
31	Great Currasow	*Crax rubra*	A	1	1
32	Greater Hornbill	*Bucerros bicornis*	BH,AS,A	6	-
33	Greater sulfur Crested Cockatoo	*Cacatua galerita*	BH	1	-
34	Green jungle Fowl	*Gallus varius*	BH	1	-
35	Green-Winged Dove	*Chalcophaps indica*	A	1	-
36	Green-winged macaw	*Ara chloroplera*	AS	1	-
37	Helmeted Guineafowl	*Numida meleagris*	A,CW	2	-
38	Hill Myna	*Gracula religiosa*	BH	1	-
39	Java sparrow	*Padda oryzivora*	CW	1	-
40	Lesser Fish Eagle	*Ichthyophaga humilis*	BH	1	-
41	Lesser Sulphur Crested Cockatoo	*Cacatua sulphurea*	CW	1	-
42	Little Corella	*Cacatua sanguinea*	CW	1	-
43	Lovebird	*Agopornis fisheri*	CW,BH,A	4	-
44	Magpie Robin	*Copsychus saularis*	BH	1	-
45	Malayan Peacock-Pheasant	*Polyplectron malacense*	BH,BA	2	-
46	Mandarin Duck	*Aix galericulata*	A	1	-
47	Milky Stork	*Mycteria leucocephala*	A	1	-
48	Moluccan cockatoo/ salmon-crested cockatoo	*Cacatua moluecencis*	AS	1	-
49	Mountain Peacock-Pheasant	*Polyplectron inopination*	BH	1	-
50	Nicobar Pigeon	*Caloenas nicobarica*	A	1	-
51	Oriented Pied Hornbill	*Anthracoceros albirostris*	BH	1	-
52	Ostrich	*Struthio camelus*	Savanna	2	-
53	Painted Stork	*Mycteria cinerea*	L,BH	2	-
54	Pigeon	*Columba*	CW,AS	3	-
55	Pin-tailed Parrot Finch	*Erythuraprasima*	BH	1	-
56	Pink-backed Pelican	*Pelecanus rufescens*	A	1	-
57	Rainbow Lory	*Trichoglossus haematodus*	CW	1	-
58	Red Junglefowl	*Gallus gallus*	A	1	-

59	Rhinoceros Hornbill	*Buceros rhinoceros*	BH,A	3	-
60	Scarlet Macow	*Ara macoa*	CW	1	-
61	Spotted billed pelican	*Pelecanus rufescens*	L	1	-
62	Silver Pheasant	*Lophura nycthemera*	A,BH,BA	4	-
63	Spotted wood-owl	*Strix seloputo*	BH,AS	2	-
64	Storm's Stork	*Ciconia stormi*	L	2	-
65	Swan Goose	*Anser cygnoides*	L	2	-
66	Turkey	*Meleagris gallopavo*	CW	2	-
67	White Bellied Sea Eagle	*Haliaetus leucogaster*	BH,AS	2	-
68	White Cockcatoo	*Cacatua alba*	BH	1	-
69	White rumped Shama	*Copsychus malabaricus*	BH	1	-
70	Wreathed Hornbill	*Aceros undulates*	A,BH	2	1
71	Wrinkled Hornbill	*Aceros corrugatus*	A	1	-
		Total		116	4

A= Aviary
BH= Birdhouse
CW= Children's world
AS= Animal show
BA= breeding area
L= Lake

TABLE 3: Percentage of fecal bird specimens positive for *Cryptosporidium* oocysts according to location

No.	Location	Total no of fecal specimens collected	No. of specimens positive for *Cryptosporidium*	Percentage positive
1	Animal Show	13	-	0
2	Aviary	37	2	5.4
3	Bird House	35	1	2.8
4	Breeding area	5	-	0
5	Children's World	18	-	0
6	Lake	8	1	12.5

TABLE 4: Biodata of the bird handlers examined for *Cryptosporidium* oocysts

No.	Name	Age (years)	Gender	Race	Location	Result (presence of *cryptosporidium* oocysts)
1	J	26	M	Malay	Bird house	negative
2	S	27	F	Malay	Bird house	negative
3	M K	21	M	Malay	Bird House	**positive**
4	M E	25	M	Malay	Bird House	negative
5	Z	19	M	Malay	Bird House	negative
6	N B H	20	F	Malay	Children's world	negative
7	M Y	52	M	Malay	Children's world	negative
8	M A M S	20	M	Malay	Children's world	negative

DISCUSSION

Cryptosporidiosis affects human and animals which include reptiles, mammals, birds, amphibians, and fish. Studies have confirmed the presence of *Cryptosporidium* oocysts in pets, domestic and wild animals (Sterling and Arrowood, 1978, O'Donoghue, 1995, Lim and Ahmad, 2001). Since the first detection of *Cryptosporidium* in birds in 1929 by Tyzzer (Sterling and Arrowood, 1978, Current, 1989), many studies have been carried out to determine the presence of *Cryptosporidium* oocysts in various species of birds. However, there are very few studies which have ascertain the naturally occurring cryptosporidiosis in wild birds. Prevalence rate of 5.9% to 27.3% have been demonstrated in flocks in the United States (Lindsay and Blagburn, 1990).

Previous study carried out in 2004 at Kuala Lumpur National Zoo showed 6 species of birds were positive with *Cryptosporidium* oocyst in their feces. The positive species included Wrinkled Hornbill (*Aceros corrugatus*), Great Argus Pheasant (*Argusianus argus*), Black Swan (*Cygnus atratus*), Swan Goose (*Anser cygnoides*), Marabou Stork (*Leptoptilos crumeniferus*) and Moluccan Cockatoo (*Cacatua moluccencis*). These birds were located in the aviary and lake and the Moluccan Cockatoo was routinely used as a show bird (Rohela et al. 2005).

This present study showed a prevalence of 3.4% (4 out of 116) of *Cryptosporidium sp.* in bird's feces. The prevalence rate has decrease

compared to the previous study which was conducted 2 years ago at the same sites. In this study, *Cryptosporidium* oocysts were detected in the feces of the Wreathed Hornbill (*Aceros undulates*) and Great Currasow (*Crax rubra)* from the aviary, Bushy-crested Hornbill (*Anorrhinus galeritus*) from the bird house and Common Peafowl (*Pavo cristatus)* from the lake.

Although the prevalence of *Cryptosporidium sp.* in birds at the Kuala Lumpur National Zoo has decreased to 3.4% compared to the previous study, the birds recorded positive in this study were different from the ones that were positive in the previous study. All 4 types of birds that were positive with *Cryptosporidium* oocysts were not found to be positive before. This finding indicates that *Cryptosporidium* infection might be spreading to other species of birds in the zoo. In both the previous and present studies, birds that were found to be positive for *Cryptosporidium* were all asymptomatic. It is very likely that these birds were not quarantined or separated from the other birds that do not harbour the parasite because they did not present any symptoms therefore facilitating the transmission of *Cryptosporidium* oocysts to the other birds.

In terms of location, a comparison between the two studies showed that the aviary and lake were still contaminated with *Cryptosporidium* oocysts whereas location that was positive in 2004 which was Animal Show was negative for *Cryptosporidium* in this present study. On the other hand, the

location that was previously negative in 2004 which was birdhouse was found to be positive in this present study.

Although there were three locations (ie. aviary, birdhouse and lake) in this present study that were positive for *cryptosporidium,* the probability of birds acquiring cryptosporidiosis might be higher in the lake because birds could get infected not only via contaminated food or close contact but also through contaminated water as well. This postulation is confirmed with the findings in this study whereby birds at the lake showed the highest percentage of positive samples for *Cryptosporidium* oocysts (12.5%) followed by the birds in the aviary (5.4%) and (2.8%) at the bird house. Birds at the animal show, breeding area and children's world were found to be negative for *Cryptosporidium* oocysts.

There are a few contributing factors to the high percentage of prevalence of avian cryptosporidiosis in the lake. Birds at the lake are not caged and therefore are free to mix with other birds or fly to any location. They might even be able to fly out of the zoo compound. This will facilitate the transmission of *Cryptosporidium* to other areas inside or outside the zoo compound. The implication of birds in the lake being infected is great simply because the lake water may be contaminated with *Cryptosporidium* oocysts-laden feces and this could be a source of transmission to other birds, animals and human who come in contact with the contaminated water. Many studies and waterborne outbreaks have implicated contaminated water as a source of

infection of cryptosporidiosis (Xiao *et al,* 2004, Current, 1989, O'Donoghue, 1995). The findings of this study warrants for future systematic study to look into the quality of water from the lake in order to determine the association of waterborne cryptosporidiosis in a zoological setup.

Occurrence of *Cryptosporidium* oocysts in the aviary is probably due to cross contamination. This is because the birds at the aviary are always being relocated from one cage to another. As an example, an asymptomatic infected bird from cage A might be transferred to cage B. Therefore if cage A is not washed properly it might still be contaminated with the feces of the previously infected bird that was transferred. When a new bird free of infection is placed in cage A, there is a chance for it to acquire the infection via the contaminated feces. This scenario enables the transmission of the *Cryptosporidium* oocysts to other birds if it is not controlled properly.

This study has discovered that one of the bird handlers who were stationed at the bird house was infected with cryptosporidiosis. This person does not only manage the birds in the bird house but he also manages the birds at the aviary and lake. As indicated earlier, all these locations that this bird handler is working were positive with infected birds. Therefore it is difficult to conclude where he acquired the infection from or whether he was the source of infection to the birds. This is because of the three avian *Cryptosporidium* sp. (ie. *C. meleagridis, C. baleyi, C galli*), only *C. meleagridis* can infect both humans and birds (Xiao *et al.* 2004). Only with the

employment of molecular techniques can this dilemma be determined through speciation or genotyping.

CONCLUSION

A comparison between a study carried out in 2004 and the present study conducted on the birds at National Zoo, Kuala Lumpur highlighted that there was a decrease in the prevalence rate in the birds however there seemed to be an indication that cryptosporidiosis might be spreading to other species of birds and to other locations in the zoo which was not previously documented. This study also discovered the probable association of cryptosporidiosis among birds and their bird handlers. However, conclusive remarks can only be done after the confirmation of speciation found in birds and bird handlers through molecular identification.